

**WHAT IS CLAIMED IS:**

1. A method for generating a population of variant sequence modules in bacterial cells, said method comprising:

- 5 (a) transferring a donor vector into a target bacterial cell capable of homologous recombination, wherein
- (i) said donor vector comprises a donor recombination module comprising, in the following order from 5' to 3': a first donor DNA sequence and a second donor DNA sequence, and
- 10 (ii) said target cell comprises a target vector comprising a target recombination module comprising, in the following order from 5' to 3': a first target DNA sequence; a negatively selectable marker; and a second target DNA sequence, wherein said first donor DNA sequence is homologous to said first target DNA sequence, and said second donor DNA sequence is homologous to said second target DNA sequence; and
- 15 (b) selecting for a population of target cells which do not contain the negatively selectable marker,

so that a population of a variant sequence modules in bacterial cells is generated.

20 2. The method of claim 1, wherein the donor vector further comprises a conjugative transfer sequence.

25 3. The method of claim 2, wherein the donor vector is present in a donor bacterial cell, and said transferring comprises conjugative transfer.

4. The method of claim 1, wherein said transferring is by transformation of the donor vector into the target cell.

30 5. The method of claim 3 or 4, wherein the donor vector is a suicide vector.

6. The method of claim 1, wherein the target vector is integrated into the bacterial cell genome.

35 7. The method of claim 1, wherein the donor vector is transferred into the target cell via a phage particle.

8. The method of claim 1, wherein the negatively selectable marker comprises a conditionally lethal sequence, and selecting for a population of target cells in step (b) comprises selecting against said conditionally lethal sequence.

5 9. The method of claim 1, wherein: i) the target vector further comprises a reporter gene sequence downstream of the second target DNA sequence; ii) the negatively selectable marker is a polar insert sequence which prevents expression of the downstream reporter gene, such that deletion of said polar insert results in expression of the reporter gene; and iii) the step of selecting for a population of target cells which do not contain the  
10 negatively selectable marker comprises selecting for expression of said reporter gene.

10. The method of claim 1, wherein the negatively selectable marker in the target recombination module comprises a unique restriction endonuclease recognition site.

15 11. The method of claim 1, wherein selecting for the a population of target cells which do not contain the selectable marker comprises amplifying DNA of the cells to determine whether the negatively selectable marker is absent from the cells.

20 12. The method of claim 1, in which the donor vector further comprises a positively selectable marker.

25 13. A method for generating a population of a variant sequence modules in bacterial cells, said method comprising:

- 30 (a) transferring a donor vector into a target bacterial cell which is capable of homologous recombination, wherein:
- (i) said donor vector comprises a donor recombination module comprising, in the following order from 5' to 3': a first non-functional fragment of a selectable-marker; a first donor DNA sequence; and a second donor DNA sequence;
  - (ii) said target cell comprises a target vector comprising a target recombination module comprising, in the following order from 5' to 3': a second non-functional fragment of a selectable-marker; a first target DNA sequence; and a second target DNA sequence,

35 wherein said first donor DNA sequence is homologous to said first target DNA sequence, and said second donor DNA sequence is homologous to said second target DNA sequence, and recombination

between said first non-functional fragment of a selectable-marker and said second non-functional fragment of a selectable-marker results in a functional selectable marker; and

- (b) selecting for a population of target cells which contain the functional selectable marker,

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so that a population of a variant sequence modules in bacterial cells is generated.

14. The method of claim 13, wherein the donor vector is present in a donor bacterial cell, and said transferring is by means of conjugative transfer of the donor vector from the donor cell to the target cell.

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15. The method of claim 13, wherein the donor vector is transformed into the target cell.

16. The method of claim 14 or 15, wherein the donor vector is a non-replicating plasmid.

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17. The method of claim 13, wherein the target vector is integrated into the bacterial cell genome.

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18. The method of claim 13, wherein the donor vector is present in a phage particle and said transferring comprises infecting the bacterial cell with said phage particle.

19. The method of claim 13, in which the donor vector further comprises a positively selectable marker.

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20. The method of claim 19, further comprising prior to step (c):  
(e) selecting for a population of target bacterial cells comprising the positively selectable marker of the donor vector.

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21. The method of claim 1 or 13, further comprising:  
(c) selecting said population of target cells of step (b) for a desired phenotype.

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22. A method for optimizing a phenotype comprising the method of claim 21, further comprising:  
(d) repeating steps (a) - (c),

wherein the target recombination module used in step (d) is derived from a target cell selected in step (c).

23. The method of claim 1 or 13, in which the donor vector further comprises a  
5 third donor sequence, located 3' to the first donor sequence and 5' to the second donor DNA sequence.

24. The method of claim 23, wherein the third donor sequence comprises a negatively selectable marker.

10 25. The method of claim 22, in which the target recombination module of step (e) is identical to the target recombination module of step (a).

09920118.073101 15 26. The method of claim 22, in which the target recombinant module of step (e) is different from the target recombination module of step (a).

27. The method of claim 1, 13, or 22, further comprising, prior to step (a), the step of mutagenizing the donor DNA vector.

20 28. The method of claim 21, further comprising, prior to step (a), the step of mutagenizing the donor DNA vector.

29. The method of claim 27, wherein the step of mutagenizing the donor vector is carried out *in vitro*.

25 30. The method of claim 28, wherein the step of mutagenizing the donor vector is carried out *in vitro*.

30 31. The method of claim 27, wherein the step of mutagenizing the donor molecule is carried out *in vivo*.

32. The method of claim 28, wherein the step of mutagenizing the donor vector is carried out *in vivo*.

35 33. The method of claim 1, 13, or 22, wherein the donor vector is a suicide vector.

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34. The method of claim 21, wherein the donor vector is a suicide vector.
35. The method of claim 1, 13, or 22, wherein the bacterial cell is an *E. coli* cell.
- 5 36. The method of claim 21, wherein the bacterial cell is an *E. coli* cell.
37. A kit useful for directed assembly of a target DNA molecule comprising in one or more containers
- 10 (a) a donor vector useful for directed gene assembly of a target DNA molecule, said donor vector comprising a donor recombination module comprising, in the following order from 5' to 3': a first donor DNA sequence and a second donor DNA sequence, and
- 15 (b) a cell which is capable of homologous recombination; and
- 20 (c) a double-stranded DNA vector useful for directed assembly of a target DNA molecule of interest, wherein said vector contains a target vector comprising a target recombination module comprising, in the following order from 5' to 3': a first target DNA sequence; a negatively selectable marker; and a second target DNA sequence, such that said first donor DNA sequence is homologous to said first target DNA sequence, and said second donor DNA sequence is homologous to said second target DNA sequence.
38. The kit of claim 37, wherein the cell of (b) comprises the double-stranded DNA vector of (c).
- 25 39. The kit of claim 37, wherein the cell is an *E. coli* cell.
40. A donor library comprising a plurality of donor vectors, said donor vectors comprising in the following order from 5' to 3': a first donor DNA sequence and a second donor DNA sequence, such that said first donor DNA sequence is homologous to a first target DNA sequence of a target gene of interest, and a second donor DNA sequence is homologous to a second target DNA sequence of a target gene of interest, wherein said donor vectors are suicide vectors.
- 30 41. The donor library of claim 40 wherein the members are arrayed.
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42. The donor library of claim 41 wherein the members are arrayed in an 8 x 12, 16 x 24, or a 32 x 48 matrix.

43. An archived module comprising a variant sequence produced by the method  
5 of claim 1 or 13.

44. A computer readable medium having a database recorded thereon in  
computer readable form, wherein said database comprises one or more module profiles and  
wherein each module profile describes a phenotype in a directed gene assembly assay, and  
10 wherein each module profile is associated with a particular recombinant vector in a  
particular target cell.

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